

Ligation Protocol

SOURCE: <https://www.neb.com/protocols/1/01/01/dna-ligation-with-t4-dna-ligase-m0202?device=pdf>

1. Set up the following reaction in a microcentrifuge tube on ice.
(*T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.*)

COMPONENT	20 µl REACTION
10X T4 DNA Ligase Buffer*	2 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Nuclease-free water	to 20 µl
T4 DNA Ligase	1 µl

2. * *The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.*
3. Gently mix the reaction by pipetting up and down and microfuge briefly.
4. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.
5. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours(*alternatively, high concentration T4 DNA Ligase can be used in a 10 minute ligation*).
6. Heat inactivate at 65°C for 10 minutes.
7. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.